

DOCKET NO: 249976US0

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :
TSUTOMU FUJIMURA : EXAMINER: LEITH, PATRICIA
A.
SERIAL NO: 10/791,753 :
FILED: MARCH 4, 2004 : GROUP ART UNIT: 1655
FOR: SKIN AGING-PREVENTING OR :
IMPROVING AGENT

DECLARATION UNDER 37 C.F.R. 1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

I, Tsutomu FUJIMURA, hereby declare as follows:

1. I have been employed by -Kao Corporation for 20 years as researcher in the field of biological science.
2. I am a graduate of Meiji Pharmaceutical University and received my Doctor of Pharmacy degree in the year 2009.
3. The following experiments were performed by me or under my supervision.
4. The following experiments demonstrate that the expression level of Rho kinase is not enhanced by agar-agar.
5. The effect of agar-agar on the expression level of Rho kinase in fibroblasts has been investigated. Agar-agar (Sigma, Cat. no. A1296-100) was dissolved in warm DMEM to prepare a 0.1% (evaporation solid residue %) solution. The solution was sterilized by filtration with a sterilizing filter and then diluted to 0.01% and 0.002% (evaporation solid

residue %). Preparation of fibroblast cultures, administration of the agar-agar solutions to the fibroblasts and detection of the expression level of Rho kinase were conducted as described in the Examples of the above-identified application. The control sample in the table below is prepared as in Table 2 of the above-identified application.

As shown in the table below, the expression level of Rho kinase was not enhanced by agar-agar.

	Concentration (evaporation solid residue %)	Rho kinase relative expression (%)
CONTROL		100
agar-agar	0.002%	89.36
agar-agar	0.01%	91.31

6. The claimed method provides advantageously increased levels of expression of Rho kinase or MLC kinase compared to the control (the protein expressed in the youngest cells (Passage number 7) taken as 100%).

7. Under penalty of perjury under the law of the United States of America.

Signature

Tsutomu Hyimura

Date

Nov. 13 2009